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IMMUNOLOGY (1975) Vol.28 No.6 pp1061-1066
Dictionary of Immunology (1989, Macmillan Press)-
Rosen et al p146

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(54) Enhancement of neutralising antibody activity

(57) Neutralising antibody activity is enhanced by exposure of the antibody to an enhancing factor comprising or extracted from human or animal saliva or an enzyme for the digestion of cabohydrates which is not inimical to the neutralising antibody, for example amylase or lysozyme, in an antibody activity enhancing amount.

A METHOD OF ENHANCING NEUTRALISING ANTIBODY ACTIVITY IN HUMAN OR ANIMAL SERA OR IN OTHER BODY SECRETIONS

One of the major strategies by which infections are combated in human or non-human multi-cellular hosts is by neutralisation of the infectivity of the micro-organism by neutralising antibody. This type of antibody elimates or 'neutralises' a discernable or identifiable effect of the micro-organism; for example, the ability of viruses to kill cells is eliminated when the virus particle comes into contact with neutralising antibody. This is clearly, therefore, an important function of antibodies in human or animal subjects.

This invention relates to an in vitro method which allows enhancement of this activity which comprises exposure of the neutralising antibody to an enhancing factor comprising or extracted from human or animal saliva or an enzyme for digestion of carbohydrates which is not inimical to the neutralising antibody, in an antibody activity enhancing amount. As examples of such enzymes there may be mentioned amylase-or lysozyme-like enzymes.

In certain experiments, neutralising activity was increased at least 500 fold

The expression "antibody activity enhancing amount" used in this specification means an amount of the enhancing factor above a threshold amount at which no enhancement is observed and below an amount at which there is no further enhancement of neutralising antibody activity. These limits will vary for different enhancing factors but for amylase and lysozyme lie within the range of 0.2 mg to 2.5 mg. and 0.5 to 10.mg per ml. of reaction mixture respectively. For human saliva and to an equal volume of an appropriate dilution of antibodies there is

a progressive increase in enhancing activity from saliva from a dilution of 4:1 with decreasing dilution 1 in 4. Concentrated eg. freeze dried saliva is more effective than diluted.

There are many applications of this invention; as examples, globulins which are intended for use in passive immunisation prior to their administration may be treated to enhance their specific activity, in other words to enhance their level of neutralising antibody activity per unit of administered immuno-globulin; alternatively in acute life-threatening infections, for example, herpes encephalitis, blood can be withdrawn from the subject, the serum treated according to the invention and subsequently with significantly enhanced activity.

This invention therefore represents a novel method of enhancing antibody activity and a novel therapeutic approach to control of serious microbial infections of human and animal subjects.

The following is presented as an example of a process which will enhance neutralising antibody activity against pseudorabies virus. This is a virus which normally effects pigs and is of considerable veterinary importance; rarely it effects human subjects although scattered cases are reported in the literature. This virus has been chosen in the example because human saliva generally does not have antibodies to this virus and therefore the enhancement effect is not masked by the fact of introducing into the serum neutralising antibodies that are already present and will themselves be enhanced in the saliva.

Hyperimmune serum prepared in rabbits was diluted 1 in 100 in

phosphate buffered saline and added to an equal volume of:-

1. Human saliva which had been heated at 56°C for 30 minutes;

2. Amylase (obtained from Sigma Laboratories) at a concentration of 0.2mgm per ml;

3. Lysozyme (obtained from Sigma Laboratories) at a concentration of 0.15mgm per ml; or

4. Phosphate buffered saline (control).

These mixtures were stood for 24 hours at 37°C and the resulting hyperimmune sera were tested in a virus neutralisation test against pseudorabies virus. The virus neutralisation test was carried out by adding equal volumes of each serum to pseudorabies virus at a concentration of approximately 10^5 pfu/ml and allowed to stand for 4 hours; samples were removed and titrated for residual virus infectivity.

The results are shown in Table 1.

There was a significant enhancement of neutralising antibody activity when the antiserum was treated with saliva, amylase or lysozyme. The antiserum that was simply mixed with phosphate buffered saline provides a control value of antibody activity.

Table 1

	Virus neutralisation (log_{10}) at 4 hr
Saliva	0.0
Amylase	0.0
Lysozyme	0.0
Anti-pseudorabies serum ($^1/_{100}$) with	
Saliva	1.60
amylase	1.30
lysozyme	1.30
phosphate buffered saline	0.40

CLAIMS

1. An in vitro method for enhancement of neutralising antibody activity which comprises exposure of the neutralising antibody to an enhancing factor comprising or extracted from human or animal saliva or an enzyme for digestion of carbohydrates which is not inimical to the neutralising antibody, in an antibody activity enhancing amount as herein defined.

2. A method according to claim 1 wherein the enzyme is amylase or lysozyme or an amylase-or lysozyme-like enzyme.

3. A method according to claim 2 wherein the enzyme is used in an amount within the range of from 0.2 mg to 2.5 mg per ml for amylase and 0.05 to 10 mg per ml lysozyme.

4. An anti-serum with enhanced neutralising antibody activity whenever prepared by a method according to anyone of claims 1 to 3.

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